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Understanding strain diversity in *Fusarium oxysporum* f. sp. *cubense* and history of introduction of 'tropical race 4' to better manage banana production

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Abstract

Fusarium wilt of banana is caused by 35 different strains or genotypes of *Fusarium oxysporum* f. sp. *cubense*. VCG 01213, so-called 'tropical race 4', is just one of six distinct strains that can attack Cavendish, but it is much more aggressive on Cavendish than strains known earlier in Australia and South Africa. New plantations established in the 1990s in peninsula Malaysia and Sumatra soon succumbed. VCG 01213 was later found to be common in village banana plants in those areas. It is only one of eight strains present in the villages. It is not a new mutant strain but is only newly recognised as unique. The combination of many strains and many different cultivars in mixed plantings allows sufficient banana production for home use. Yet attempts to grow Cavendish in plantation fail within a few years of establishment. Commercial Cavendish production in Taiwan, where strain VCG 01213 is present, is possible only by planting partially resistant Cavendish mutants in rotation with paddy rice and ratooning only twice. Even so, losses continue and costs are high. Permanent commercial plantations are no longer economic in areas where VCG 01213 is found. However, surveys indicate this pathogen is not ubiquitous. VCG 01213 was recently introduced into the Northern Territory of Australia, where eradication was attempted. This involved investigation of source, elimination of entire banana fields, isolation of sites, no replanting and tight quarantine. The situation here is not confounded by 'village banana plants' and is simplified by isolation and distance from the country's main banana-growing areas. In the Philippines and China, the disease now occurs in plantations situated within large areas of commercial banana production. The threat was at first not taken seriously and strain identification was delayed. Spread has occurred very rapidly in South China and less so in the Philippines. The VCG 01213 pathosystem is virtually unstudied, and its biology and epidemiology unknown. The results of experimental studies in Sumatra on incidence, treatment with endophytes and antagonists, cassava rotation, timing of infection, root invasion and breeding for resistance are reported. Rotation with cassava appears promising.

INTRODUCTION

We now know that Fusarium wilt of banana is caused by many different strains or genotypes of *Fusarium oxysporum* f. sp. *cubense* (Foc). Since the strains are clonal and have evolved with different wild banana species in different places, they have unique characteristics of pathogenicity and, presumably, of ecological properties. We know little about their differences beyond finding by default differences in varietal host range and

aggressiveness. All surmises are based on circumstantial evidence, as no planned experiments at the field level have ever been conducted.

So-called 'tropical race 4' (TR4) is a catchy but inaccurate name. The term 'race' is not appropriate for these pathogens, as each strain is unique and can be identified. The race concept is based on three clonal triploid host differentials, a largely unworkable system. In any case, a given host may be attacked by many strains that are different epidemiologically and in host range, yet they are combined into a single race (Ploetz, 2006; Gerlach et al., 2000). I recommend dropping the concept of race as envisioned by Stover (1962) and using 'strain' instead. Strains are identified by VCG analysis and given numbers. VCG stands for "vegetative compatibility group", which can be evaluated easily (Ploetz, 1990; Leslie and Summerell, 2006). Some isolates fit no yet-constructed VCG and have been given genotype numbers on the basis of DNA fingerprint data (Bentley et al., 1998). In the future, new isolates not fitting an existing VCG could be typed via AFLP or RFLP until a VCG is constructed for them. In any case, a genetically specific and identifiable isolate (strain) becomes a non-confounded base for studies of host-range and epidemiology. Any "lumping" into larger categories should follow only after such studies show identical behavioural attributes for two different VCG strains.

TR4 is just one of six distinct strains that can attack Cavendish (AAA genome) in *both* the tropics and subtropics. It is best designated as strain 01213 (zero-twelve-thirteen). This strain is much more aggressive on Cavendish than the so-called 'subtropical race 4' strains from Cavendish known earlier in Australia (0120, 0121, 0129, 01211), the Canaries and South Africa (0120 only) and the Philippines (0122). I do not consider the widespread Indian strains VCG 0214/5, which devastated 'Gros Michel' (AAA genome), to be competent on Cavendish since their very rare recovery from Cavendish has been on plants very stressed.

ORIGIN

Most strains of Foc attacking banana originated in different places in tropical Asia, where all of some 30 plus wild species of banana also originated and evolved. Strain 01213 probably originated in the Malay peninsula and in Sumatra (these were connected during the last Ice Age and at different times earlier). I consider its widespread occurrence within Indonesia (Sulawesi and Irian New Guinea excepted) and its presence in Taiwan to be from dispersal over time in corms of the village banana cultivars 'Pisang Berangan' (AAA genome), 'P. Rastali' (AAB genome) and 'P. Raja' (AAB genome).

We think of strain 01213 as a Cavendish strain. "Race 4" was first designated in Taiwan by Su in 1977 on the basis of wilt in Cavendish (Su et al., 1986). Strain 01213 was first designated on the basis of samples sent from Taiwan to the USA in 1989/90 (R.C. Ploetz, pers. commun.). The next 01213 samples were from village banana plants collected by Stover in Sumatra in 1992 and from village banana plants in Malaya in the same year, but not from Cavendish. It was only gradually realised in 1993-94 that Cavendish was very vulnerable in those areas after commercial Cavendish plantations were established for the first time in Malaya, Sumatra and Java. Plants were promptly attacked and plantations soon abandoned, as the rapid spread of the disease could not be stopped. Only two Cavendish plantations still remain in these countries and these are in a precarious state. The threat from strain 01213 also prevents the establishment of new plantations.

Samples from the Malaysian part of Borneo and the many samples from the potential Cavendish-growing land in Central Sulawesi did not reveal the presence of

strain 01213 (Ploetz and Pegg, 2000; N. Moore, pers. commun.). Thus, the presence of this strain is not ubiquitous in Malaysia and Indonesia. Indeed, even in Sumatra there is a location where Cavendish ('Valery') has been grown for many years without becoming diseased.

The key question is how large an area in the centre of origin of Fusarium wilt contains the native strain 01213. It may be a very small area, with widespread occurrence now only in villages where it was taken along with the corms of banana varieties, as they were disseminated over the years. It is useful to realise that each banana variety was once only a single plant and that the same varieties have been propagated and distributed over time to all the villages of Southeast Asia. It is also useful to appreciate that these varieties largely went south and east into Malaysia and the archipelago islands now known as Indonesia, many from India. Thus, they carried northern mainland strains of Foc and picked up new ones. But the reverse hardly occurred. Inferences can be taken from the sampling data on clones affected by the various strains in Malaya and Sumatra (Tables 1 and 2).

Is 01213 present in forest and countryside beyond the villages themselves? We do not know, as this has never been investigated. It would be easy to do by planting tissue culture-derived plants beyond the villages on upland soils. At present, villagers try new sites, but always with corms from their villages.

Since wild banana species are never diseased in situ (for the exception, see Bentley et al., 1998), their widespread healthy occurrence in an area is not an indicator of absence of strain 01213 or of any other strain pathogenic to a domesticated clone. However, wild banana species are susceptible to various strains when they are removed from their native habitat to soils containing strains not present in their homeland. Their susceptibility was known, but not appreciated nor understood, in the early 1960s when the United Fruit Company assembled a large collection on a Honduran soil containing the strains that took out 'Gros Michel' (mainly strains from Java (0120) and the Philippines (0126), but also from India (0124, 0125)). Many wild accessions were very susceptible (pers. observ.).

The potential implications of susceptibility/resistance of wild species for deducing where different strains were present in native soils and for decision-making in breeding approaches were not realised. The great diversity of strains was not then known, but Vakili (1965) hypothesised that resistance in wild species was related to evolution with Foc. I realised the true implications only in the 1990s when extensive explorations always revealed healthy wild bananas in situ, yet diseased clones in villages. This anomaly required an explanation.

I recently tested wild *M. acuminata* ssp. *malaccensis* on soil heavily infested with 01213 in Sumatra and it remained healthy, whereas both *M. banksii* from Sulawesi and *M. sumatrana* from Central Sumatran highlands were severely diseased. This means that where these grow wild and remain healthy, 01213 is not present. By more testing of wild species and subspecies from different sources, one could determine much about absence or presence of various strains in native soils.

One further piece of evidence was gleaned from observations in Malaya where tissue culture-derived Cavendish banana plants were interplanted with new oil palm on large tracts of land, which had been under oil palm for 40 years. On such land, no villages were present, but the wild banana *M. acuminata* ssp. *malaccensis* had been present earlier. By 36 months, Fusarium wilt incidence was 3% and the strain was typed as 01213. Cases occurred at random. In the much more susceptible 'P. Berangan', which

was also planted, incidence was 30%. This information suggests that strain 01213 was native and an endophyte with wild *M. acuminata* ssp. *malaccensis*, at a low population level. The strain would also appear to have survived without banana under oil palm for over 40 years.

MANAGEMENT OF VILLAGE BANANAS IN INDIGENOUS TR4 AREAS

In Sumatra and Malaya, banana plants are grown in yards in villages or *kampongs*. Many varieties may be present in clumps in a single yard. The soil is usually rich from household detritus. As jungle is cleared and new villages established along new roads, sucker-corms are taken to new locations, along with their parasites, pathogens and insect pests. This has been occurring over millennia and it continues in the same way. Since Fusarium wilt symptoms do not appear in suckers, they are considered healthy and thus strains of Foc that were present at the old site are moved to the new site. This not only occurred locally, but also from island to island, from country to country and from continent to continent. The strains of India, one strain from the Philippines (0126) and one from Java (0120) became intercontinentally spread as clones originating in those places were dispersed. But some strains were left behind (01213, 0122) or remained unidentified in 'Gros Michel' and do not attack the replacement Cavendish, and thus, remain hidden.

When specimens of Foc were collected extensively in Sumatra and Malaya in the early 1990s, Fusarium wilt was commonly seen in villages (N. Moore and I. Buddenhagen, unpublished information). Now, however, wilt is seldom seen in villages in southern Sumatra (I. Buddenhagen, unpublished information). The explanation is simple. People abandon their favourite, highly susceptible varieties of 'P. Berangan' and 'P. Rastali' and continue to grow other less susceptible varieties, such as 'P. Nangka' (AAA genome), 'P. Rejang' (AA genome) and 'P. Berlin' (AA genome). That is the extent of 'management'. Where villagers still attempt to grow 'P. Berangan' on newly cut forest land in northern Sumatra for the Medan market, they use clean-looking, but infected suckers, so the disease continues and is readily seen there. 'P. Berangan' in markets is now rare and very expensive. In more remote areas, such as in Central Aceh in 'Forest Reserve' areas, Foc was apparently not infecting the few corms introduced and one even sees healthy 'Gros Michel'. This indicates that 01213 and other strains are not native there, but no experimental plantings have ever been made with tissue-culture plantlets and pasteurised polybag-grown plants into potentially "clean" ground. Of considerable interest is a 'Dwarf Cavendish' clone (or clones) that occurs widely in villages in Sumatra and Java, usually as a single clump. It has never been seen with Fusarium wilt, even when near other clones affected by 01213. Their behaviour under plantation conditions has not been tested.

MANAGEMENT OF BANANA PRODUCTION IN A TR4 AREA OF OLD INTRODUCTION

In Taiwan, Cavendish wilt has been known for many years. I saw it there in 1966. It was long considered to be the same as the 'subtropical wilt' in Australia and South Africa, but it is now known to be mainly caused by strain 01213. Also present is strain 0121, which can also attack Cavendish (Ploetz, 1990). Both strains are present in the old Giant Cavendish clone called 'P. Buai' (AAA genome) grown in highland Sumatra. Malaya also has both of these strains. The Fusarium strains and some of the banana varieties in Taiwan must have come from one of these areas long ago.

A review of the disease indicates an explosive epidemic from a point source, beginning in 1967, and a 'mutation' was suggested (Su et al., 1986). However, no one looked at local banana plants in that area nor at the source of the Cavendish corms (Hwang, 1985). We now know that spread of strains of Foc generally is via corms of village banana plants. The suggested explanation of mutation for a new outbreak is no longer tenable, nor needed, to explain the disease's history on Taiwan.

The earliest attempts to manage Fusarium wilt in Cavendish were carried out at the Taiwan Banana Research Institute near Pingtung by S.C. Hwang. Tissue-cultured plants were planted in fields where the pathogen occurred and survivors selected for further tissue-culture propagation. These somaclonal variants resisted the pathogen to varying degrees, and the best were multiplied for commercial purposes. Plants are now ratooned only once or twice and followed by a rotation with paddy rice before replanting. In addition, farmers moved banana production with tissue-culture plantlets to new fields. The pathogen was not generally present and it became epidemic only from inoculum build-up in the first Cavendish plants affected. In one upland area, a 6-year rotation is now used with normal 'Giant Cavendish' followed by pineapple and papaya. However, Fusarium wilt recurs and remains a problem (K.S. Soung, pers. commun.). Hwang's agronomically best mutant clone is less resistant than his earlier mutant clones, which are inferior agronomically. Nevertheless, mutant clones have enabled continued production in Taiwan for the Japanese market. Climatic conditions are subtropical to tropical, with a cold season. Also, the diverse village bananas of the Asian deep tropics are not present.

MANAGEMENT OF TR4 AND BANANA PRODUCTION IN AREAS WHERE IT HAS BEEN RECENTLY INTRODUCED

Strain 01213 has been introduced recently to the Darwin area of the Northern Territory in Australia (1997), Mindanao, in the Philippines (2006) and Southern China (2000). The year indicates first recorded occurrence, but introduction could have been 5 to 15 years before. In each country, a different approach to control was applied.

In the Northern Territory of Australia, where no wilt had occurred on Cavendish previously, symptoms were immediately suspect and disease experts from Queensland quickly identified the strain involved. A strict quarantine was imposed. Whole fields were eradicated and fenced, and a quarantine research station was established. The idea was to eradicate the introduction, but to keep a research site. This is a dry tree-savanna region where no villages or other banana plants were present, and some 1600 km from the important banana growing areas of Eastern Queensland. Despite efforts to minimise spread, plants at 14 farms became affected and 10 farms ceased production. Where the initial infection was untreated at first, the farm rarely survived commercially beyond 3 to 4 years. It was definitely a new introduction to one site only; exactly how it spreads between separated farms is only speculation (Walduck and Daly, personal discussions). It is probable that Javanese workers, who were brought in several years before to the farm where the first outbreak occurred, introduced the pathogen. (I found a single dying clump of 'Rastali' (syn. 'Silk') present in a yard there in 2002.)

In contrast to the Australian approach, Cavendish wilt in the Guangdong area of southern China was ignored at first and is now epidemic. It is probable that corms brought from Taiwan introduced the pathogen. However, without in-depth field analysis, there is no way to reconstruct source, manner of spread or management options. In 1992, there was no Fusarium wilt in the many plantations, but there was considerable disease in a banana germplasm collection near Guangzhou (I. Buddenhagen, pers. observ.). No

sampling was permitted and no analysis was carried out, or at least made public. Chen Houbin sent a sample from Zhongshan in Guandong to Queensland for determination in November 2000, which was typed 01213.

TR4 was recently identified in Mindanao in the Philippines, which is now the second largest banana exporter in the world. It is present at scattered locations within the Cavendish-growing areas on different company farms. Silva and Meredith of Del Monte first found Cavendish wilt in 1974, which was later determined to be due to a new, endemic strain (0122). Although some research was conducted by Del Monte, this strain, which is known only from Mindanao and mainly from Cavendish, proved to be mild and research was dropped. In 2002, Silva saw severe cases of early wilt in a Dole plantation and suspected TR4. Since no expertise was present in the Philippines to conduct VCG analysis, the strain was identified as 01213 in South Africa only in 2006. There has been no real effort to elucidate the manner of introduction and spread nor to determine the extent and location of cases. Since Filipinos either administered or worked in the Cavendish plantations in Malaya and Sumatra, which developed severe TR4 disease, it may not be too surprising that TR4 should now be found in Mindanao. However, an alternative source may have been banana plants in villages in the northern arm of Sulawesi. TR4 occurs there and considerable traffic for generations has occurred over the few hundred miles separating Manado from Davao. As in China, the problem was first ignored. In addition, Moko bacterial wilt was confused with Fusarium wilt with many cases being counted as Fusarium wilt instead of Moko. Since no one in the country can identify strains, little can be accomplished. So 'management' of TR4 Fusarium wilt is uncertain and in the hands of various company managers, as is the production system itself. This varies from company to company, but all are patterned after experience in Central America, from whence the export business was introduced in the 1960s. Little is known about the Fusarium strains in the Philippines. Many isolates do not fit a known VCG and the Australians just gave genotype numbers to those sent for identification (Bentley et al., 1998). Much more is known about banana Fusarium strains in Indonesia, Malaysia and other Asian countries, than in the Philippines.

Although 01213 was reported as present in India (Gerlach et al., 2000), the single case found was in a recent introduction of an AA into the germplasm collection at Trichy and the disease has not become established (I. Buddenhagen, pers. observation). Thus, it should not be considered to be present in India yet. However, the readiness with which it spread to China, the Philippines and Australia should be of major concern to the world banana trades.

MANAGEMENT OF BANANA PRODUCTION IN A PLANTATION IN SUMATRA WHERE TR4 MAY BE INDIGENOUS

There is only one banana plantation left in Indonesia, that of Nusantara Tropical Fruit (NTF) in South Sumatra. This plantation was started by Filipinos working for Del Monte, Mindanao, as a joint venture in about 1991. The Cavendish variety first used was 'Valery', which was initiated from tissue-culture plantlets. In 1993-94, it was being devastated by Fusarium wilt. From investigations undertaken by N. Moore and I. Buddenhagen, only two hypotheses were possible for the widespread outbreak: (1) The pathogen was already widespread in the soil even though no village or wild bananas had been present, or (2) the non-pasteurised soil mix used in the polybags had contained the pathogen, which was then distributed over the whole plantation in the transplanting operation. This latter explanation seemed more likely, as soil had been used from riverine

areas near an abandoned village (Moore et al., 2001). The Filipinos had never seen rampant Fusarium wilt, but they applied the same techniques for control as had been used in Mindanao where they knew only the mild strain 0122. Ditches were dug around affected plants or groups of affected plants. Diseased plants were chopped and packed into black plastic bags. These control practices probably aided the spread of the disease. Del Monte soon closed down its operations with a considerable loss.

NTF held on, got advice and clones from Hwang in Taiwan and continued. A large tissue-culture and nursery operation was started and is still operative. Under the technical leadership of Hardono Nugroho, a new planting system was tried: double rows, closely planted, but spaced widely (some 8 m) from the next double row. All suckers were removed as they appeared and only the plant crop was harvested, with elimination of all plants after harvest with glyphosate. A new double row was then planted in the middle, with 3-month old nursery plants from polybags. This constituted the second crop. The original sites are then planted again for the third crop. Thus there is, in effect, a 15-20 month rotation of planting sites.

An enormous and expensive tissue-culture and nursery operation is required as well as considerable precise planning of many operational procedures. Two mutant clones from Taiwan are grown, 'DM2' ('GCTCV-119') and 'CJ20' ('GCTCV-218', 'Formosana'). 'DM2' is more resistant, but has an inferior bunch and develops high mat. 'CJ20' is better agronomically, but is more susceptible. The practice is to grow 'CJ20' on low incidence blocks for two or more cycles and then switch to 'DM2' when incidence reaches 15-25%. Disease incidence in the 'DM2' that follows 'CJ20' is usually only 5-10%.

ENDOPHYTIC *FUSARIUM OXYSPORUM*

Fusarium oxysporum was isolated from roots of healthy wild bananas in Sumatra beginning in 2004, and subsequently from roots of wild bananas in Java and Sulawesi. These were from various sub-species of *M. acuminata. Fusarium* spp. were isolated earlier from roots of wild bananas collected in peninsular Malaya by Adeline Ting (2003). VCG studies have not yet been conducted on any of these isolates. This is preliminary work and sampling has been limited. However, these findings, combined with the evidence of wild species being always healthy in situ, yet susceptible to foreign strains, support my hypothesis of the origin of pathogenic strains of Foc as only *F. oxysporum* endophytes in wild banana roots in different areas. This suggests they are not pathogens in their host of origin, but can become pathogenic to introduced foreign germplasm.

Strain 0120 was one of the strains that led to the demise of 'Gros Michel' as an export plantation variety in Central America. However, it was unable to cause disease in Cavendish planted in Central America. Yet, 0120 is the strain causing Fusarium wilt of Cavendish in the Canaries and South Africa. This anomaly led to the hypothesis that other strains from 'Gros Michel' that could not cause disease on Cavendish were present in these soils in Central America and that they possibly invaded banana roots and acted as protective endophytes. The results of two experiments devised to test this hypothesis at NTF were inconclusive, but there were indications that incompatible Foc strains did not protect against pathogenic 01213.

These inconclusive results led to re-analysis of the original hypothesis of protection by Foc strains non-competent on Cavendish. First, we do not know which endophytes are present in roots of Cavendish in Central America on old 'Gros Michel'-abandoned soils. This should be investigated. But we do know that at least one strain is

still alive under Cavendish in Honduras where a newly bred variety was planted and promptly became diseased by Fusarium (Caid, pers. commun.). No susceptible host ('Gros Michel') had been present for more than 40 years. Thus it is highly probable that Foc is an endophyte there in Cavendish. Or there may be alternative endophytic hosts (Hennessy et al., 2005).

More damaging to the idea that applied endophytes of any kind should work is a consideration of banana root development. After planting to the field, new roots develop from within the corm from the central cylinder edge, one above the other. These grow through corm cortex and emerge into the soil. The plant develops a large root system of new roots, which would not contain any applied endophyte, unless the endophytes applied earlier could grow proximally into the corm and mix there with the tissues generating the new roots, which is considered unlikely. Moreover, by the time of transplanting, the tips of many primary roots are dead and more are broken in transplanting. The plant must quickly produce new roots to survive.

DISEASE BIOLOGY AND PATHOGEN ECOLOGY

Disease biology and pathogen ecology are basically unstudied for the Cavendish/strain 01213 pathosystem. The management of this disease is based on speculations, inferences and intuition from research undertaken 50 to 60 years ago in Central America (Stover, 1962) and on experience in Taiwan (Su et al., 1980; Sun et al., 1978).

Management of Fusarium wilt is a misnomer. How to manage banana production in the face of Fusarium wilt, however, is a reality, and it is specific for each cultivar/strain pair and each soil/climate environment. Generalisations are dangerous.

In an effort to better understand disease biology at NTF, young, tissue culture-derived plants showing symptoms as early as 3 months after planting were examined in detail. Discolouration of the stele of primary roots extending into the young cormlet occurred on only one or two roots. In the young cormlet, tracheids and vessels are anastomosed early, leading to rapid movement of the pathogen followed by discoloured vascular tissue in outer leaf sheaths and to leaf lamina folding and degreening. Discoloured vascular tissue extended into petioles and even into midrib and lamina. The pathogen was isolated from the midrib and the lamina close to the midrib. Sporodochial production on the surface of these tissues was sought unsuccessfully, but this requires careful search in the humid rainy season and a positive result would be of great importance in disease epidemiology. At the time symptoms were first detected, no symptoms extended from the cormlet out into other roots. These observations, combined with isolations, indicate that many infections occur in the secondary roots, but few of these successfully become systemic to cause visible disease. Thus, the host has a great ability to block systemic infection, but this ability is not absolute in Cavendish with strain 01213. The key should be on how to enhance its normal defences. These findings led to different approaches to plant management to lessen incidence.

TREATMENT OF DISEASED PLANTS

Based on the hypothesis that disease incidence is inoculum driven and that inoculum is mainly built up within sick plants, different treatments were applied to affect this build-up. In six blocks of 7 ha each at NTF, disease cases were: (a) removed (for later burning); (b) injected with Roundup; or (c) left untouched. The more resistant clone 'DM2' was planted.

Disease incidence in the first cycle was: (a) 2.1%; (b) 3.1%; and (c) 3.8%. For the second cycle, results were: (a) 2%; (b) 3.4%; and (c) 4.1%. Removal of diseased plants thus resulted in a reduction by half of the disease incidence compared with the control. These small differences were statistically significant on the large sample base from the six blocks. Roundup injection gave intermediate results. It appears that some inoculum is returned to the soil with Roundup treatment. Exactly what happens with the inoculum as plants die with Roundup has not yet been investigated. Most cases were not clustered, indicating low root-to-root spread. Further data analysis showed that late-appearing cases (last 27 weeks) differed markedly by treatment, with prompt removal resulting in a total of 38 cases, whereas no treatment had 149 cases and Roundup an intermediate 88. These cases probably result from inoculum from earlier cases rather than from residual soil inoculum. Even so, the low incidence and thus low spread is remarkable in that plants are spaced only 1.2 meters apart in the row. A further inference drawn from the raw data is that much infection occurs between the time of transplanting and a few months of age.

The hypothesis of very early infection, with some delay before symptom appearance, led to a more careful examination of plant growth and of practices carried out from transplanting to the 3-month stage. First, breakage of roots occurs during transplanting. Second, rotted cassava peel is placed in the transplanting hole. This contains soil from wherever it was grown. Third, the planting hole often becomes waterlogged. Fourth, a flush of suckers occurs rapidly, apparently generated by the residual hormonal condition from the tissue-culture methodology. These suckers are much more abundant than with plants starting from bits or sword sucker material. These abundant suckers generate abundant roots. These suckers are unwanted and are pruned underground with a spatula tool several times in the first months. This pruning creates large wounds all around the young corm of the mother plant and it cuts an unknown number of root bases on the young mother plant, providing an ideal infection route. This underground pruning also removes a great number of healthy new growing roots, depriving the plant of some 30-40% of its feeding roots.

DISEASE BUILD-UP BY CYCLES

Disease incidence in relation to crop cycle was studied in four blocks of 7 ha planted with ‘DM2’ and ‘CJ20’ somaclonal selections from Taiwan.

For ‘CJ20’ plants, incidences in three consecutive cycles were: 7.8% - 11% - 22.1%. Diseased pseudostems were added to the block having 22% incidence in ‘CJ20’ to make a high uniform inoculum for experimental purposes. However, management then planted this experimental plot for production with ‘DM2’. Fusarium wilt occurred in 60% of these plants, a level never seen before in ‘DM2’ plots. A few rows of ‘Valery’ planted for comparison suffered a 100% loss. This work confirmed that disease incidence is inoculum driven, even for the most resistant clone.

Where ‘DM2’ followed ‘DM2’, incidence by cycle was: 2.9% - 15% - 12% - 7.3%. It appears that inoculum does not build up much under ‘DM2’ or something maintains ‘DM2’s defences.

In some blocks, the clones were alternated. In terms of plant positioning, only cycles 1 and 3 or 2 and 4 were planted on the same row site. Results by cycles were as follows in one block: ‘DM2’ 3.3% - ‘CJ20’ 11.6% - ‘DM2’ 13.7% - ‘DM2’ 7.5%. In another block, results were: ‘DM2’ 2.8% - ‘CJ20’ 14.7% - ‘CJ20’ 31% - ‘DM2’ 9.6%. It was concluded that ‘DM2’ provides enough maintenance of inoculum for a 5-6 fold increase in incidence of the ‘CJ20’ clone. ‘CJ20’ doubled in incidence when it followed a

previous ‘CJ20’ crop. Yet after a 31% incidence in ‘CJ20’, the following crop of ‘DM2’ had less than 10% of plants affected. The inference for the last set of data is that the ‘CJ20’ double row contributes inoculum to the central area for the next planting.

So, under the existing agricultural practices, the system is not sustainable with ‘CJ20’ and only the poorer clone ‘DM2’ allows continuance.

ROTATIONS AND ALTERNATE CROPS

An obvious agronomic change could be rotation or fallowing, with the expectation of lowering inoculum. Banana plantation management everywhere resists this option and NTF is no exception. But NTF’s planting design also offers possibility of interplanting a suppressive crop between the double rows, and considerable practical experimentation could be carried out on different potential crops or plant species. Sorghum, lima beans and sunhemp were introduced, which grew well inter-row. But the work was not followed up.

The NTF banana plantation is surrounded by thousands of hectares of cassava, so this is the ideal crop for a rotation trial. A single, 5-year old, 20-ha field of cassava adjacent to the banana plantation was replanted as six blocks of banana. The cassava field had been originally planted to ‘Valery’, but this had been destroyed by Fusarium wilt. Only 0.98% of ‘DM2’ plants were affected in the first cycle and 0.24% in the second. Only 2.31% of ‘CJ20’ plants became affected in the first cycle, followed by 2.30% in the second. However, Fusarium wilt incidence in the first cycle of ‘CJ20’ was 4.95% in one of the six blocks. The second cycle in this block was planted to ‘DM2’, which had only 0.33% Fusarium wilt incidence. Low incidence has been maintained in a third cycle. Thus, although surrounded by blocks having 20-30% incidence, the disease is under effective control. Interplanting with cassava in the wide space between double rows has also proven to result in much lower incidence than normal, so far, for three generations. The cassava is not harvested, but chopped and disked into the soil. This work indicates that rotation or interplanting with cassava greatly reduces incidence. The practice provided effective disease control and should be more widely tested. Research is needed to determine how cassava acts as a suppressive crop species. The present hypothesis is that inoculum is lowered under cassava. Whether this is a direct effect or an indirect one by reducing or eliminating symptomless weed hosts remains to be determined.

EARLY PROTECTION

Since a large portion of cases occurs in the first 6 months, an experiment was conducted to attempt to protect or enhance defence on young plants. Different chemicals and biologicals were tested, by application to roots on deflasking. Early results indicate some effect with Propiconazol and the biological Sonata. Much more research is needed and should be concentrated on early defence enhancement/protection experiments. In addition to root dipping on deflasking, applications should be made during the 10-week nursery polybag stage.

SUCKER PROPAGATION

Tissue-culture plantlets develop more severe Fusarium wilt than plants derived from suckers (Smith et al., 1998). In the “hot spot” block where ‘Valery’ had 100% disease incidence, ‘CJ20’ in the endophyte trial had 60-80% incidence by harvest. On those surviving until shooting, pruning was stopped in order to obtain suckers. On the plants with no symptoms through to harvest, plants were both ratooned and other suckers

were taken and transplanted down the row where Fusarium had occurred. On this first sucker generation, incidence dropped to about 25%. Incidence was thus reduced greatly, being no higher than in second-cycle normal plantation blocks. A second generation of suckers is now under test in comparison with tissue culture-derived plants for control. Early results indicate that lower incidence in second sucker-derived plants continues.

AGRONOMIC PRACTICES

At NTF, the soil is shallow with a heavy clay subsoil. No mechanical tillage is practiced. Irrigation is either drip (for the double-row system) or under-tree sprinklers (for non-moveable square planting). During heavy rains, there is surface run-off. Fusarium has been spread throughout. Practices which should be considered as potentially negative for plant health and vigour in general and which may enhance Fusarium incidence as well are several: (1) monthly underground pruning, continued through to harvest, cutting many roots; (2) hand tool removal of all surface leaf/stalk material so as to keep bare the soil around the plants, thus cutting roots close to the surface; (3) hoeing around the plants shortly after planting; (4) piling all organic matter in rows in the open space away from the growing bananas; (5) digging large holes for new plantings in the standing crop, cutting roots in the process; (6) planting in very deep in holes which become waterlogged; (7) planting nursery plants that are too old and have many dead roots; (8) applying Roundup frequently on weeds arising on the bare soil and on the banana roots at the surface near the weeds; and (9) not practicing rotations, unlike what is done in Taiwan with the same pathogenic strain.

POTENTIAL NEW VARIETIES AND PRACTICES

There are many varieties in the villages and some of these are immune to strain 01213. However, these varieties are not competitive in productivity with Cavendish. In an effort to develop a resistant and productive banana through breeding, a germplasm collection of some 150 accessions was assembled at NTF. Crossing was conducted for 2 years and interesting progeny having one immune village diploid parent have been obtained.

In the collection, agronomic handling was different from that practiced in the plantation based on experience and knowledge of fungal behaviour and disease development. No underground pruning was practiced, and sucker pruning by cutlass was kept to a bare minimum. All banana residue was chopped and kept around the plant base and beyond up to 2 m (objective is 4 inches of mulch). Rows were single spaced and plants were double ratooned. No deep holes were dug for planting and no Roundup was used to kill plants or weeds. When Fusarium wilt occured, the mother plant was taken out while the suckers were left.

After 3 years, Fusarium wilt was absent on most entries, but severe on ‘Gros Michel’ and a few other entries, including two wild species. The root systems of these plants were extensive, and many roots grew at the soil surface below the mulch, which was degrading into organic soil. Such roots do not exist under plantation management where some of the root system is removed in pruning and soil scraping. The root system of plantation-grown plants at shooting is very limited, with much root rot. The assumption is that several standard plantation practices are both deleterious to productivity and enhance Fusarium wilt.

CONCLUSIONS

The approach to Fusarium wilt control in 'Gros Michel' plantations in the Americas was first to move to clean ground and later to change varieties. In Taiwan, with Cavendish, the approach was the same, with the addition of tissue-culture plantlets and rotations, especially with paddy rice, and by the creation of new varieties (somaclonal mutants). Fusarium remains a problem even so, as no mutant exists that is both immune and comparable agronomically to Cavendish.

For the villagers in tropical Asia, the solution to Fusarium wilt has been the same. They just change varieties. If they want the best variety for local markets, they try 'clean ground' by clearing the forest. Unfortunately, they plant infected sucker-corms, just as the American plantations had done.

For plantation banana production near areas where village banana plants already have 01213, the strategy should be: (1) search for good soil that has no 01213 by planting test plots of 'P. Berangan' and start there when possible; (2) start with plantlets derived from tissue culture grown well in polybags of pasteurised well draining mix; (3) plan for rotations with cassava or paddy rice; (4) use agronomic practices that do not injure roots and that provide plenty of surface mulch; (5) make maximum use of banana tissues as mulch; (6) cut deep drains to improve drainage; (7) ratoon one, two or more times to maximum productivity and profit; (8) use several of the best clones available; and (9) consider different varieties.

The ideal solution would be to find an immune Cavendish equivalent in a village in Southeast Asia. This may exist, but is anyone looking? Otto Reinking, the great Fusarium wilt scientist sent to Asia by the United Fruit Company, found 'Valery', which was eventually used as a replacement for 'Gros Michel', in a garden in Saigon in 1926, yet it was 35 years before it was used to solve the Fusarium wilt problem there.

The dream of some conventional banana breeders is to develop a Cavendish type of banana resistant to both Mycosphaerella leaf spots and Fusarium wilt. The dream of genetic engineers is to do so by transformation. Whether this will happen remains to be seen. Such a clone would greatly upset the world banana markets and cause problems for the fungicide producing companies. The target of obtaining by conventional breeding a really good village banana that is immune to Fusarium wilt and has high *Mycosphaerella* resistance should be easy to attain.

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Tables

Table 1. *Fusarium oxysporum* f. sp. c*ubense* strains in Sumatra.

Native	Mainly in cultivars
0120	'Gros Michel', 'P. Rastali'
0121	'Oisang Buai' (Robusta), 'P. Rastali'
01213	'P. Berangan', 'P. Rastali', Cavendish, 12 others
01218	'P. Kepok'/'P. Awak'[1], 'P. Rastali'
01219	'P. Kepok'/'P.Awak'[1], 'P. Ambon', 'P. Nangka'
Not native (rare)	Mainly in cultivars
0123	'P. Awak' (from Thailand) or 'P. Rastali' ('Silk') (from
0124/5	India)

[1]Uncertain if 'P. Kepok' or 'P. Awak'.

Table 2. *Fusarium oxysporum* f. sp. c*ubense* strains in Malaya.

Native	Mainly in cultivars
0121	Cavendish
01213	Cavendish, 'P. Raja', 'P. Berangan', 7 others
01217	'P. Rastali'
012181	'P. Rastali'
Genotype 5, similar to 01217	*Musa acuminata* ssp. *malaccensis* (one site)[3]
Not native	Mainly in cultivars
0123[2]	
0124/5[2]	'P. Awak' and 'P. Rastali'
0125[2]	

[1]Uncertain if native (only one case); may be from Sumatra.
[2]Strain 0123 is Thai, strains 0124/5 and 0125 are Indian.
[3]See Bentley et al., 1998.
Table derived from Bentley et al., 1998 plus Moore data from mainly Buddenhagen and Moore collections (unpublished).